

REPLACEMENT
SHEET
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STEPS IN
PCR/RE/LDR

WILD-TYPE

MUTANT

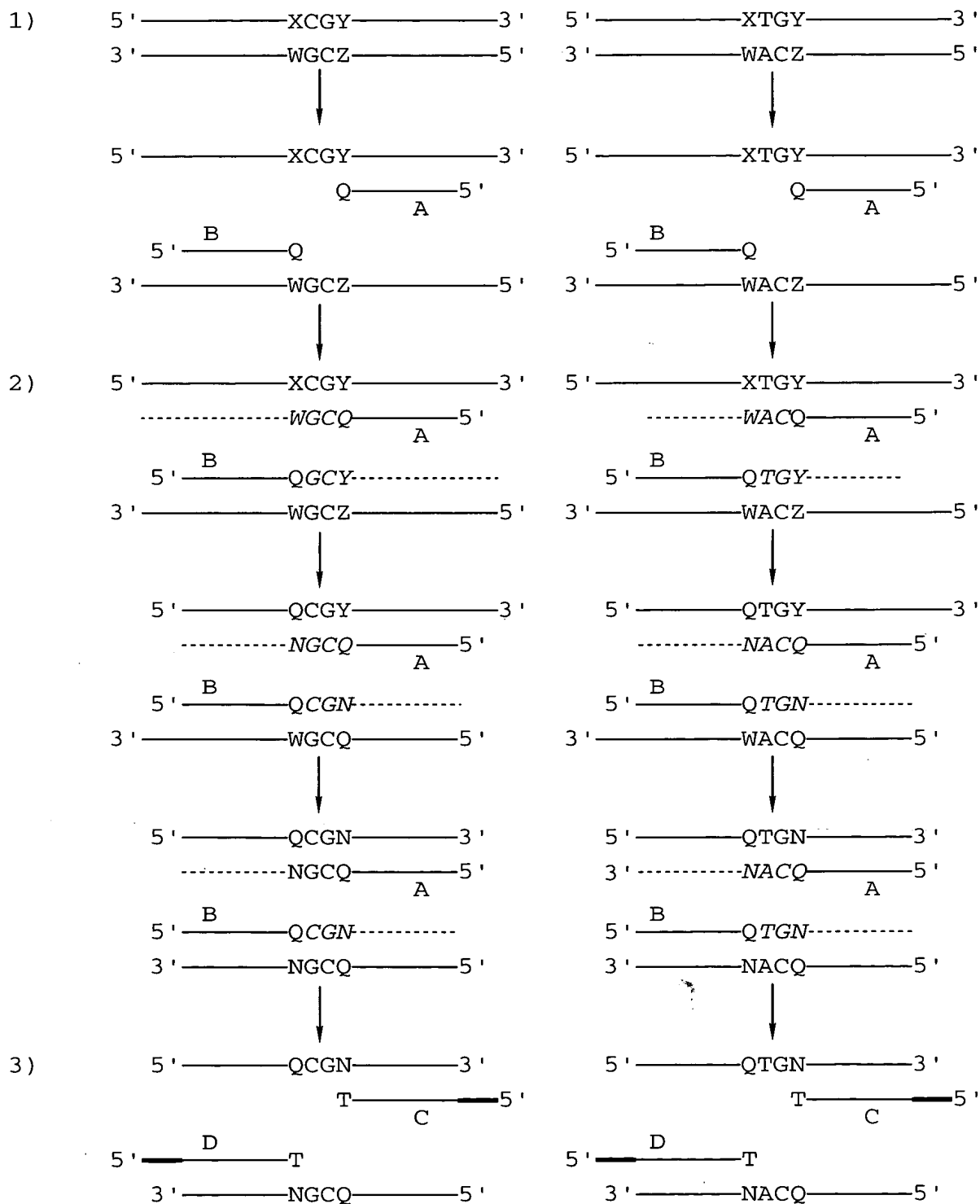
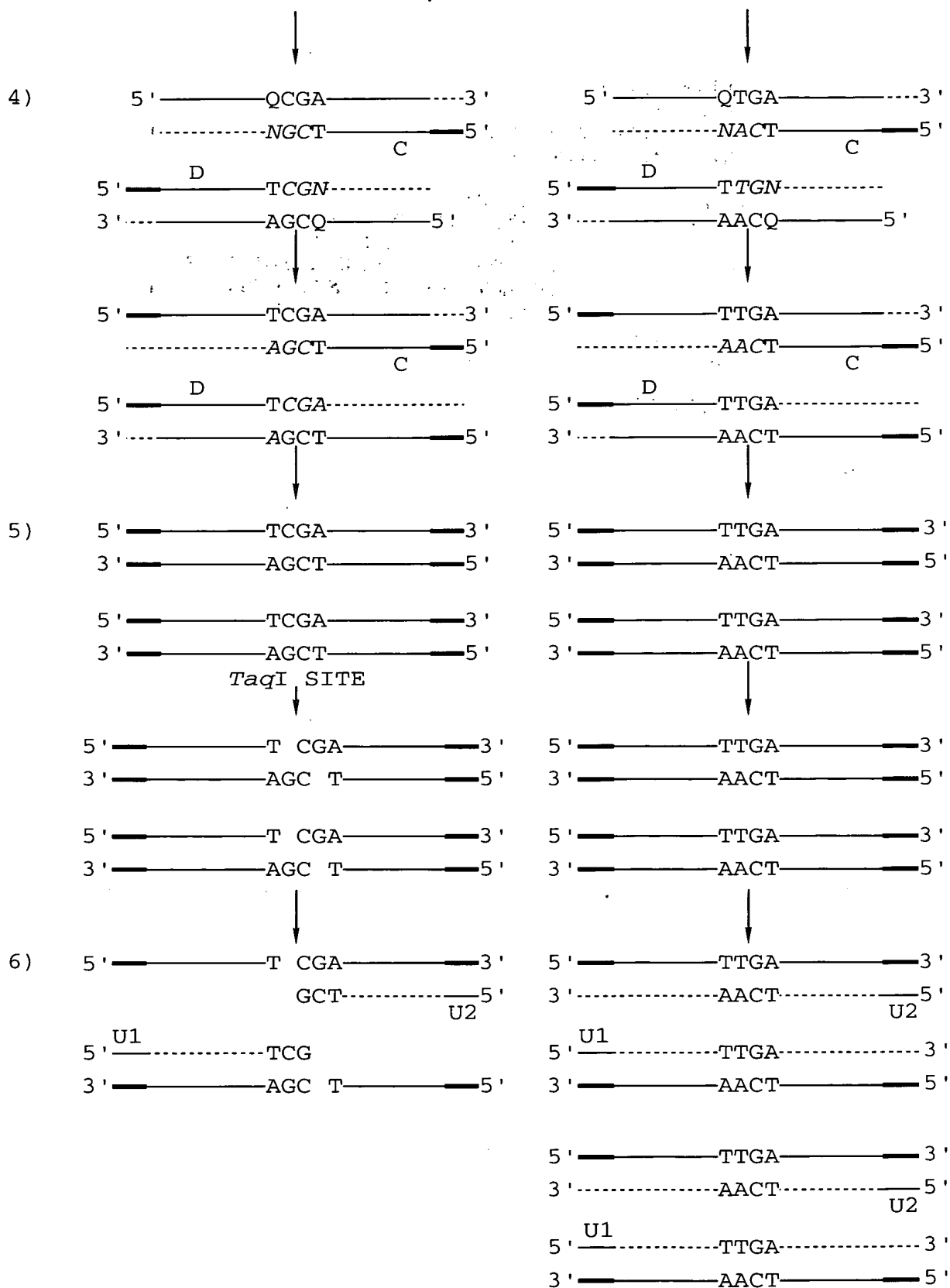


FIG. 1A





REPLACEMENT SHEET

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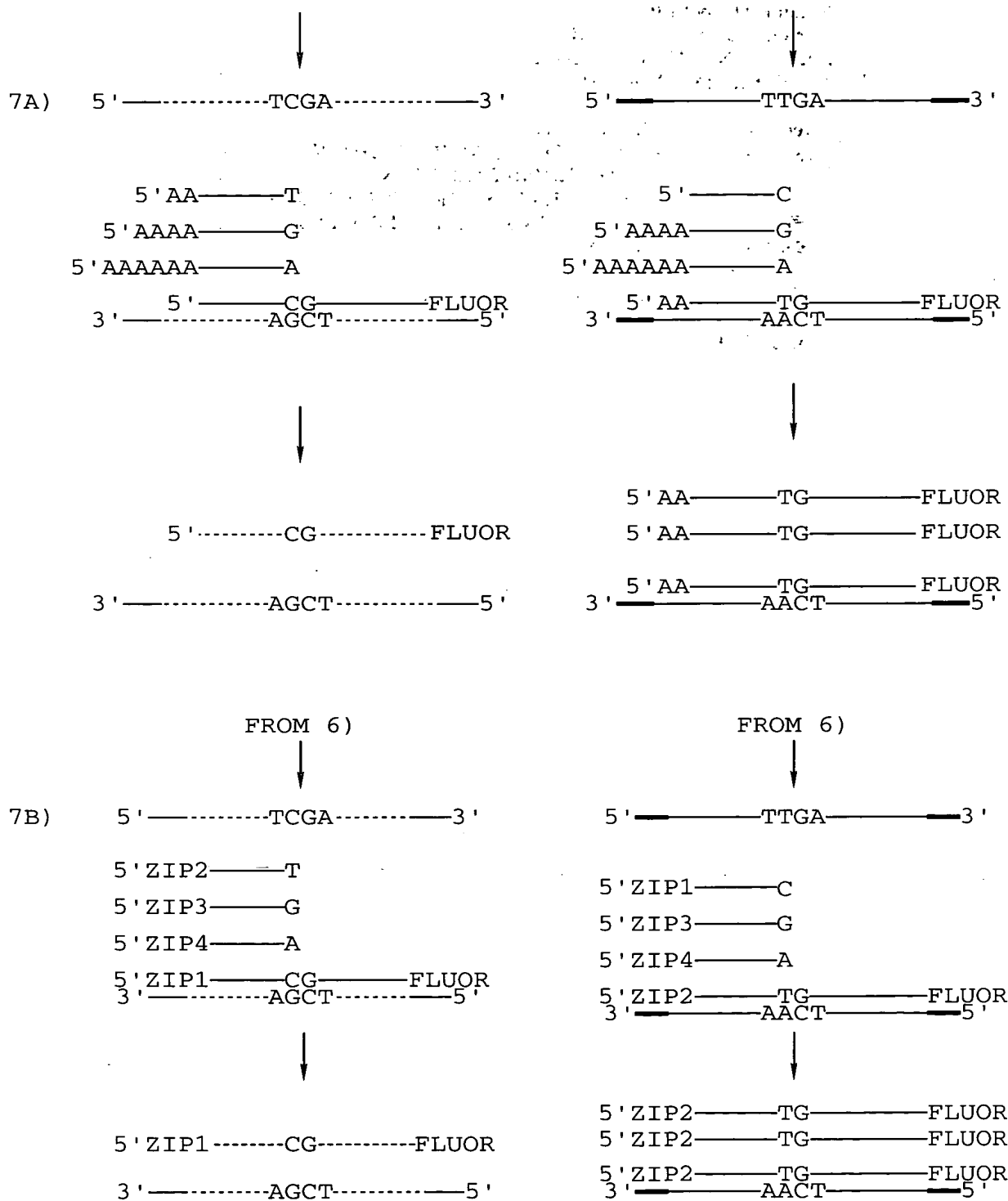


FIG. 1C



REPLACEMENT SHEET

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5' AA ——— TG ——— FL

5' ----- CG ----- FL

5' AAAAAA ——— A

5' AAAA ——— G

5' G ----- FL

5' AA ——— T

5' ——— C

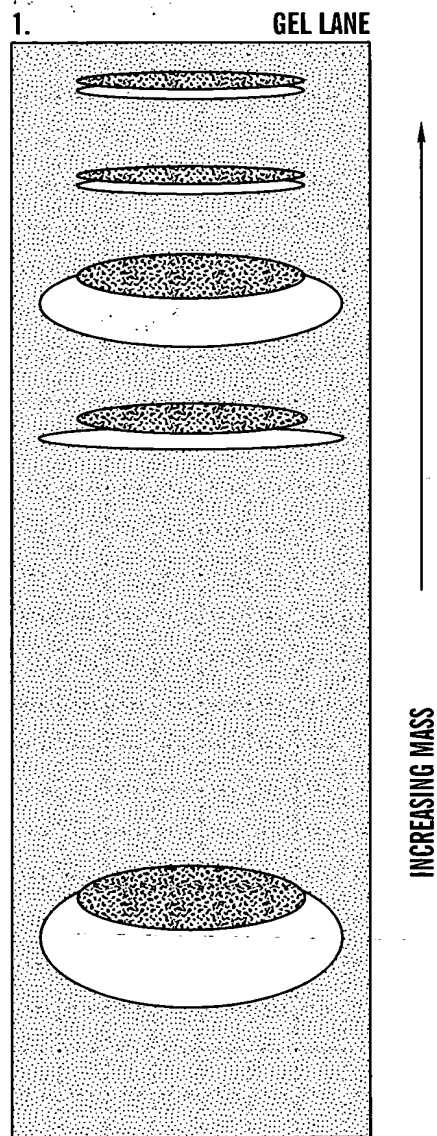
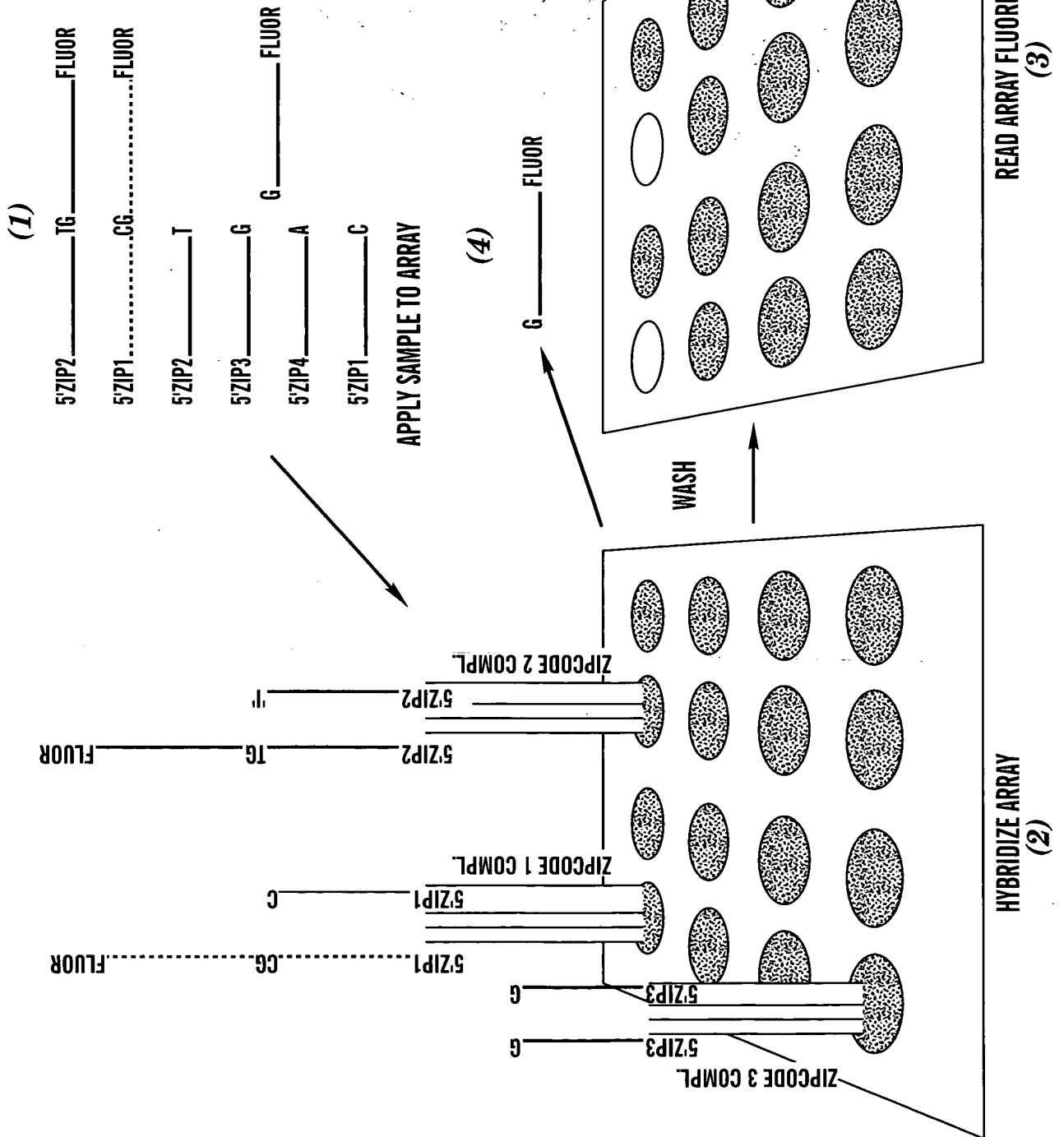


FIG. 2

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FIG. 3



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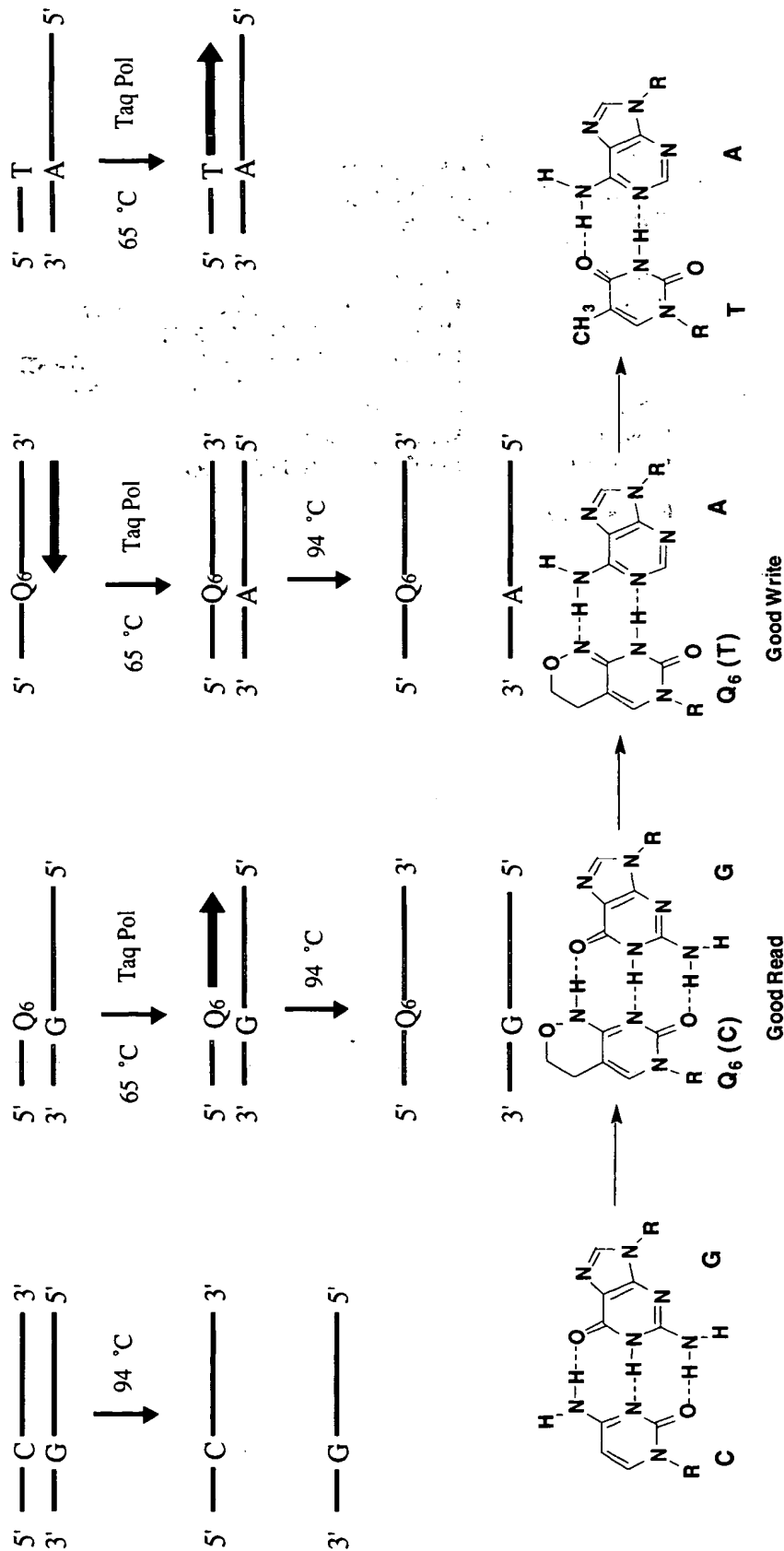
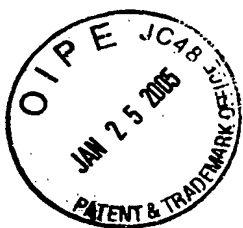
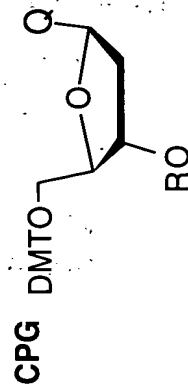
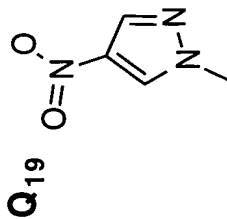
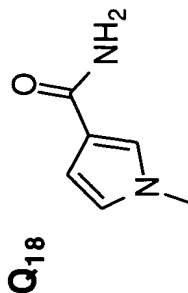
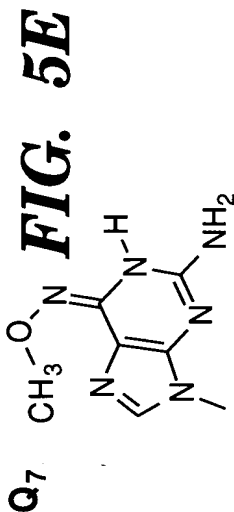
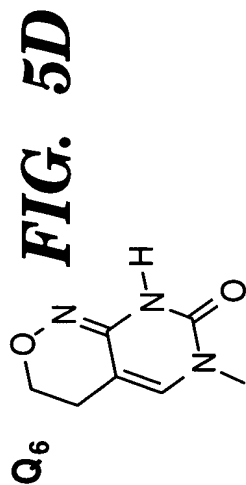
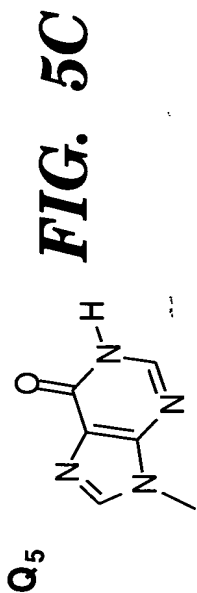
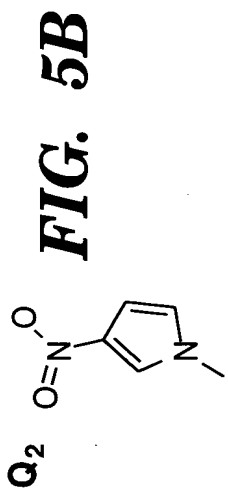
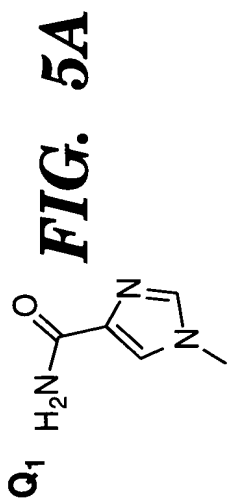


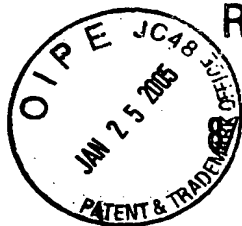
FIG. 4



REPLACEMENT
SHEET

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REPLACEMENT SHEET

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PRIMERS

codon248

Ztop CTT GGA CGA GTT CAT ACG C

P53zip248 CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG A

P53-248X T TCT TCC TGC ATG GGC GGC ATG AAX→pol

|| |||| |||| |||| |||| |||| ||||

50 bp synthetic

duplex DNA

p53-248XR

p53zip248R

Zbot

3' CA AGG AGC TAC CCG CCG TAC TTG GCC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

5' GT TCC TGC ATG GGC GGC ATG AAC CGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

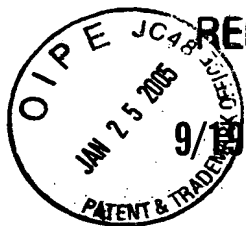
: |||| |||| |||| |||| |||| ||||

pol←X TCC GGG TAG GAG TGG TAG TAG TCT T

C GGG TAG GAG TGG TAG TAG TGC ACC GGT GGG TCA AAC G

C ACC GGT GGG TCA AAC G

FIG. 6A



REPLACEMENT SHEET

PRIMERS

codon248

CTT GGA CGA GTT CAT ACG C

CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GGC ATG AAT

p53-248Q_N T TCT TCC TGC ATG GGC ATG AAQ_N → pol

|| ||| ||| ||| ||| ||| ||| |||

50 bp synthetic

3' CA AGG AGC TAC CCG CCG TAC TTG GCC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

duplex DNA

5' GT TCC TGC ATG GGC ATG AAC CGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

: ||| ||| ||| ||| ||| ||| |||

p53-248 Q_NR

pol → Q_N TCC GGG TAG GAG TGG TAG TAG TCT T

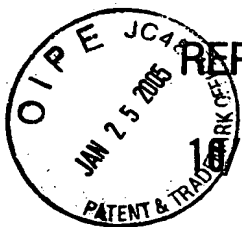
p53zip248TR

T TCC GGG TAG GAG TGG TAG TAG TGC ACC GGT GGG TCA AAC G

Zbot

C ACC GGT GGG TCA AAC G

FIG. 6B



REPLACEMENT
SHEET

LDR PRIMERS

DISCRIMINATION

COMMON

P53LDR248FCA

F-AAAAA GC ATG GGC GGC ATG AAC A

P53LDR248FCG

F-AAAA GC ATG GGC GGC ATG AAC G

P53LDR248FCT

F-AA GC ATG GGC GGC ATG AAC T

P53LDR248FCC

F- GC ATG GGC GGC ATG AAC C-...*ligase*

P53LDR248PGG

GG AGG CCC ATC CTC ACC ATC AT-block

3' (- strand)

5'

CONVERSION

...GTA TGC GCA AGG ACG TAC CCG CCG TAC TTG NCC TCC GGG TAG GAG TGG TAG TGA ACC...

PRODUCTS

FIG. 6C



REPLACEMENT SHEET

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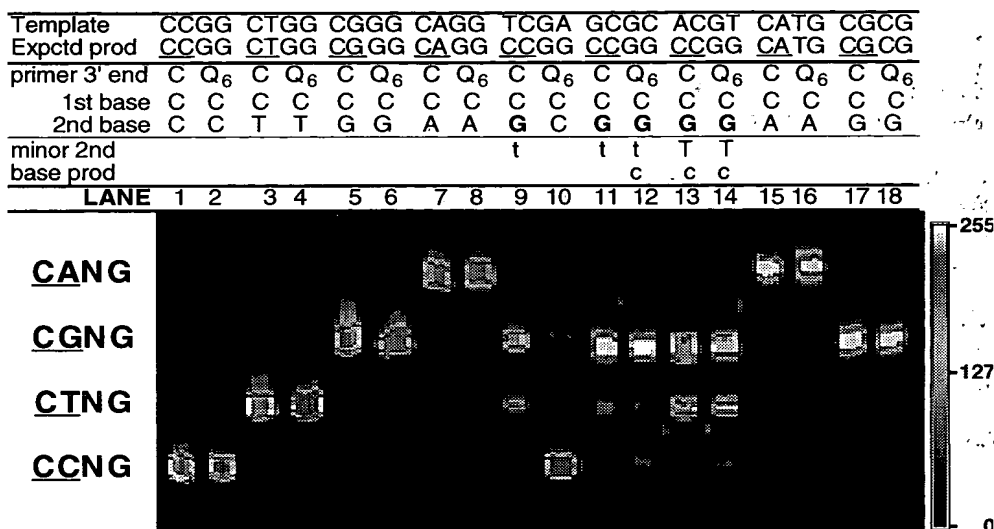


FIG. 7A



FIG. 7B



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Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	GC GC	GT GC	GG GC	GAGC	GC GC	GC GC	GC GC	GATC	GG CC
primer 3' end	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇
1st base	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	? ? ?
minor 2nd base prod		t t t	g g	A A a					

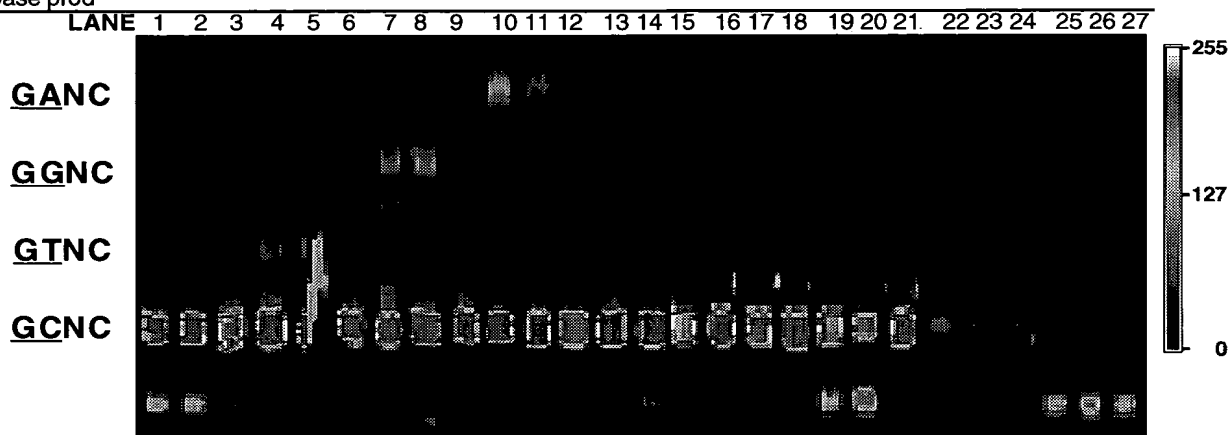


FIG. 8A

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	ACGT	ATGT	AGGT	AAGT	ACGT	ACGT	ACGT	AATT	AGCT
primer 3' end	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅
1st base	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C
minor 2nd base prod	?		?						

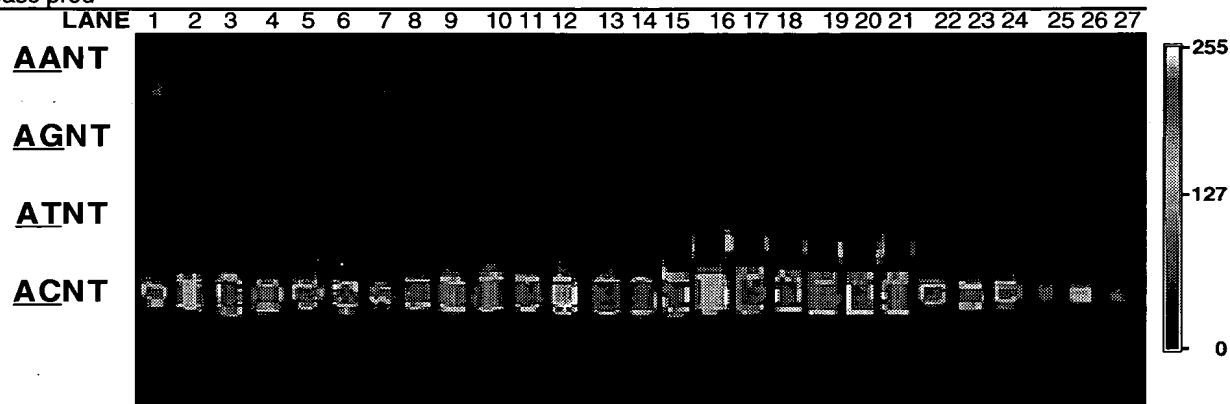


FIG. 8B



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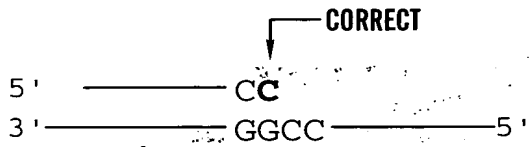


FIG. 9A

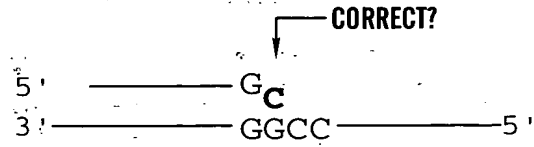


FIG. 9E

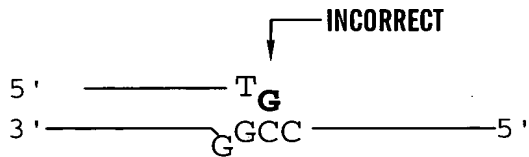


FIG. 9B

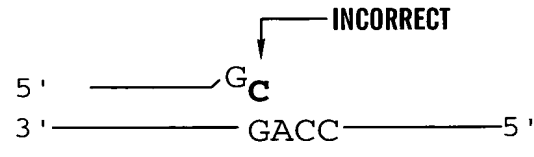


FIG. 9F

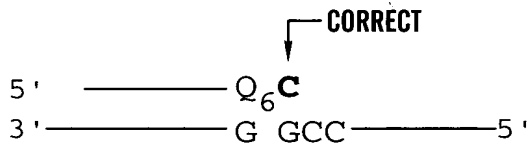


FIG. 9C

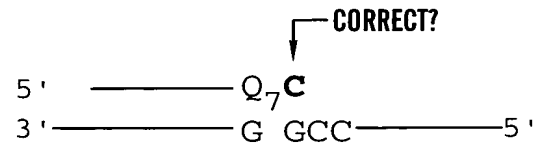


FIG. 9G

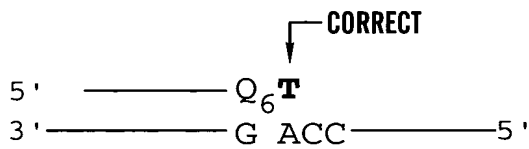


FIG. 9D

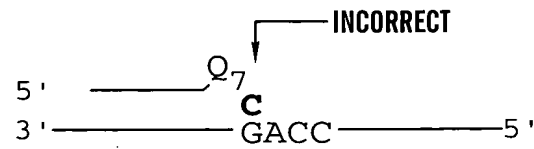


FIG. 9H



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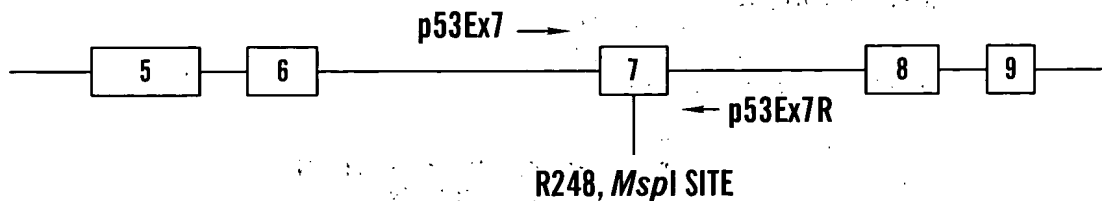


FIG. 10A

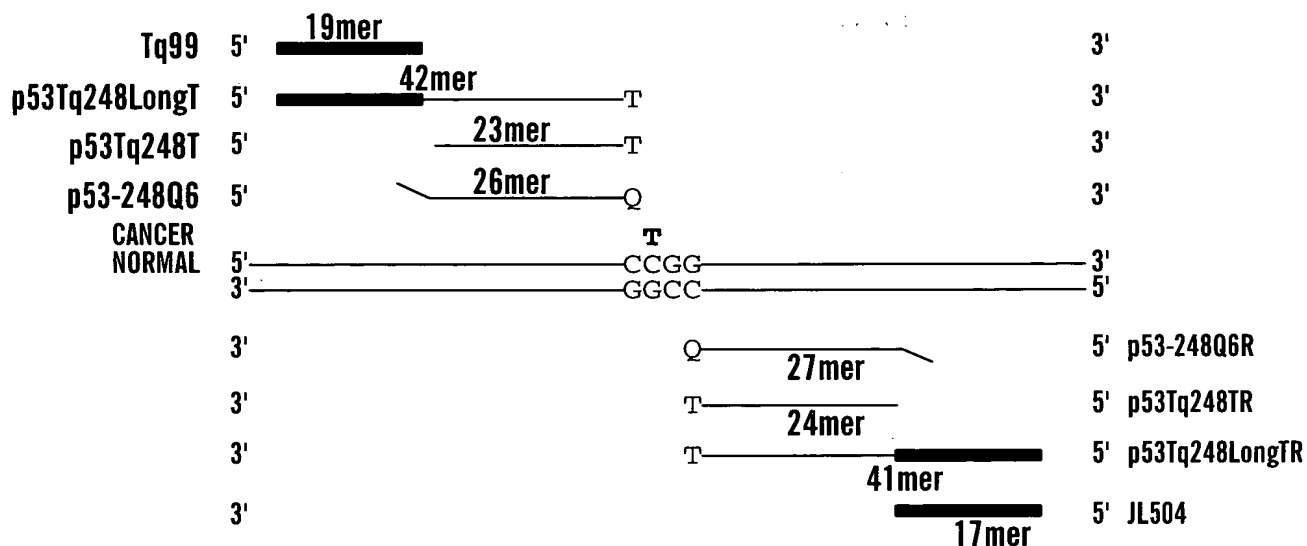
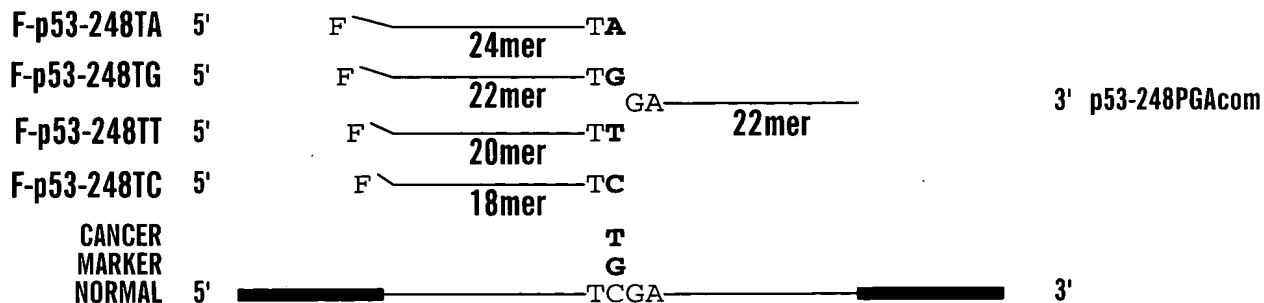


FIG. 10B



CODON 248 CONVERTED TO A *TaqI* SITE

FIG. 10C



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*Msp*I (CCGG)
codon 248

FIG. 11A

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*Msp*I (CCGG)
codon 248

FIG. 11B

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FIG. 11C



REPLACEMENT SHEET

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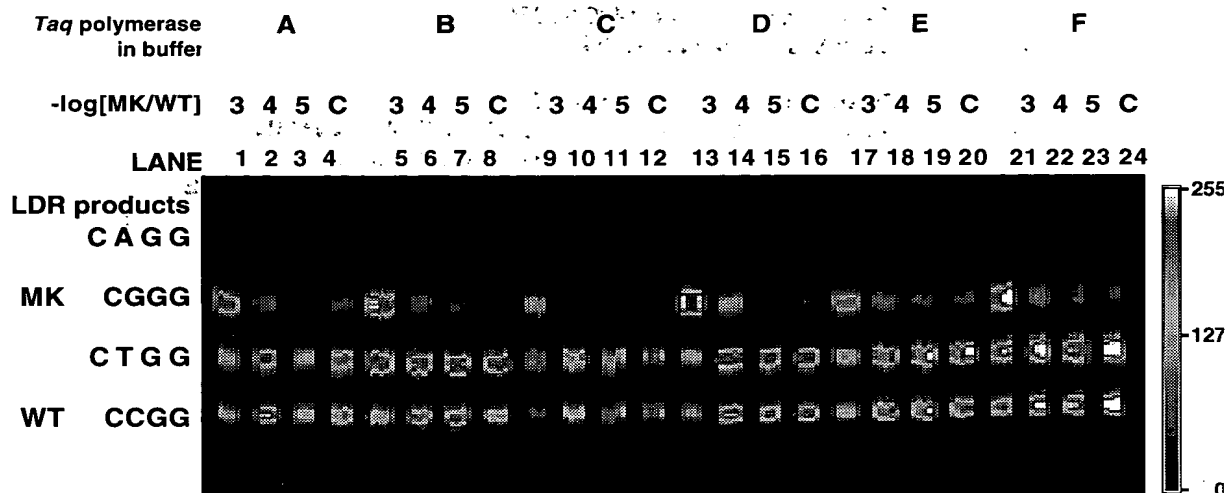


FIG. 12A

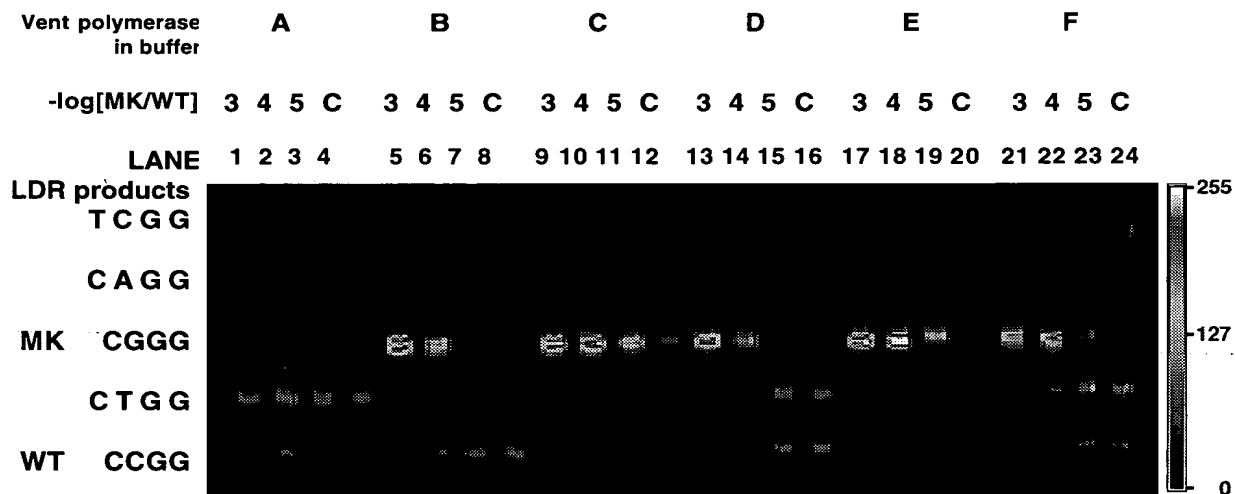
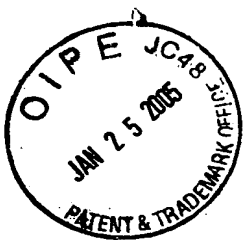


FIG. 12B



REPLACEMENT SHEET

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Conversion	C:G				Q ₆ :G				T:G			
-log[MK/WT]	3	4	5	C	3	4	5	C	3	4	5	C
LANE	1	2	3	4	5	6	7	8	9	10	11	12

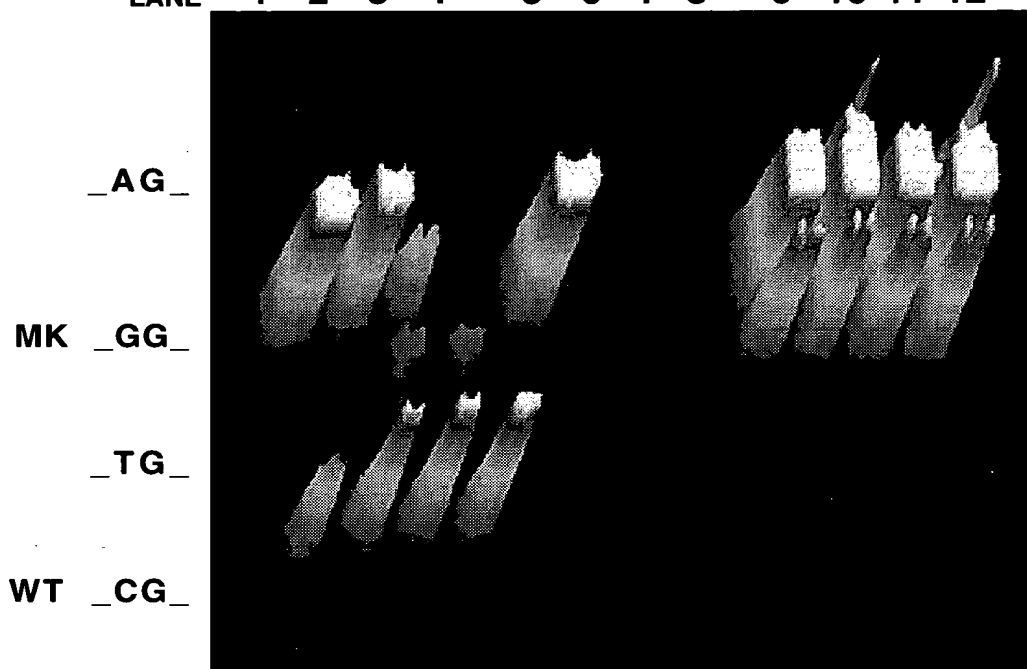


FIG. 13

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